INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/003149

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/08 C12N15/64 C12R1/91 A61K48/00 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12R A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MORENO-FLORES M TERESA ET AL: "Immortalized olfactory ensheathing glia promote axonal regeneration of rat retinal ganglion neurons." JOURNAL OF NEUROCHEMISTRY, vol. 85, no. 4, May 2003 (2003-05), pages 861-871, XP002302026 ISSN: 0022-3042 cited in the application *whole document, in particular: Abstract*	1-30
Y	WO 02/088337 A (RUBIO RODRIGUEZ MARIA PAZ ; BLASCO MARHUENDA MARIA ANTONIA (ES); CONSE) 7 November 2002 (2002-11-07) cited in the application *wwhole document, in particular: pages 8, 9 and 11*	1-30

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2004	08/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	BULCAO DE MELO BARRE

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/003149

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PAILLARD F: "Reversible cell immortalization with the Cre-lox system." HUMAN GENE THERAPY. 1 JUL 1999, vol. 10, no. 10, 1 July 1999 (1999-07-01), pages 1597-1598, XP002302027 ISSN: 1043-0342 *whole document, in particular: figure 2*	1-30
Y	SALMON P ET AL: "REVERSIBLE IMMORTALIZATION OF HUMAN PRIMARY CELLS BY LENTIVECTOR-MEDIATED TRANSFER OF SPECIFIC GENES" MOLECULAR THERAPY, ACADEMIC PRESS, SAN DIEGO, CA,, US, vol. 2, no. 4, October 2000 (2000-10), pages 404-414, XP001028604 ISSN: 1525-0016 cited in the application *whole document, in particular: Abstract *	1-30
Y	NALDINI ET AL: "Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 21, 15 October 1996 (1996-10-15), pages 11382-11388, XP002114690 ISSN: 0027-8424 cited in the application *whole document, in particular: Abstract*	1-30
Y	WESTERMAN K A ET AL: "REVERSIBLE IMMORTALIZATION OF MAMMALIAN CELLS MEDIATED BY RETROVIRAL TRANSFER AND SITE-SPECIFIC RECOMBINATION" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 17, 20 August 1996 (1996-08-20), pages 8971-8976, XP000674657 ISSN: 0027-8424 cited in the application *whole document, in particular: Abstract*	1-30

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/003149

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 629 159 A (ANDERSON DAVID J) 13 May 1997 (1997-05-13) cited in the application *whole document, in particular: column 3, line 36-column 4, line 62; column 11, lines 10-27; column 13, line 10-column 14, line 12; column 16, lines 11-17 and claims*	1-30
A	SANTOS-BENITO FERNANDO F ET AL: "Olfactory ensheathing glia transplantation: a therapy to promote repair in the mammalian central nervous system." THE ANATOMICAL RECORD. MAR 2003, vol. 271B, no. 1, March 2003 (2003-03), pages 77-85, XP002302028 ISSN: 0003-276X *whole document*	
A	RAMON-CUETO A ET AL: "OLFACTORY ENSHEATHING GLIA: PROPERTIES AND FUNCTION" BRAIN RESEARCH BULLETIN, ELSEVIER SCIENCE LTD, OXFORD, GB, vol. 46, no. 3, June 1998 (1998-06), pages 175-187, XP001157028 ISSN: 0361-9230 cited in the application *whole document*	

INTERNATIONAL SEARCH REPORT

.rnational application No.
PCT/GB2004/003149

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 14, 19, 28
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 14, 19 and 28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB2004/003149

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02088337	A	07-11-2002	ES	2190336 A1	16-07-2003
			WO	02088337 A1	07-11-2002
US 5629159	A	13-05-1997	AT	255632 T	15-12-2003
			AU	700690 B2	14-01-1999
			AU	6107796 A	30-12-1996
			CA	2222425 A1	19-12-1996
			DE	69630955 D1	15-01-2004
			DE	69630955 T2	21-10-2004
			EP	0832196 A1	01-04-1998
			ES	2211959 T3	16-07-2004
			JP	11507230 T	29-06-1999
			NO	975557 A	05-02-1998
			WO	9640877 A1	19-12-1996

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WPP288389	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/003149	International filing date *(day/month/year)* 19.07.2004	Priority date *(day/month/year)* 18.07.2003
International Patent Classification (IPC) or national classification and IPC C12N5/08, C12N15/64, C12R1/91, A61K48/00, A61P25/00		
Applicant CONSEJO SUPERIOR DE INVESTIGACIONES CIENT... et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.05.2005	09.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bulcao de Melo Barre Telephone No. +49 89 2399-8972

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/003149

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-23	as originally filed
Claims, Numbers	
1-26	filed with the demand
Drawings, Sheets	
1/7-7/7	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 27-30
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-26
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-7, 9-11, 13-19 and 21-26
	No:	Claims	8, 12 and 20 (No assessment, see section V, item 5.2)

2. Citations and explanations (Rule 70.7):

see separate sheet

SECTION I

1. Amendments (**Article 34 (2) (b) PCT**)

Amended **claims 1-26** filed with letter of 17.05.05 are considered to be allowable under **Article 34 (2) (b) PCT.**

SECTION V

2. Reference is made to the following documents:

D1: J. of Neurochemistry, Vol. 85, No. 4, 2003, pages 861-871
D2: Human Gene Therapy, Vol. 10, No. 10, 1999, pages 1597-1598
D3: Molecular Therapy, Vol. 2, No. 4, 2000, pages 404-414
D4: Proc. Nat. Acad. Sci., Vol. 93, No. 21, 1996, pages 11382-11388
D5: Proc. Nat. Acad. Sci., Vol. 93, No. 17, 1996, pages 8971-8976
D6: US 5 629 159
D7: The Anatomical Record., Vol. 271B, No. 1, 2003, pages 77-85
D8: Brain Research Bulletin, Vol. 46, No. 3, 1998, pages 175-187

3. Novelty (**Article 33(2) PCT**)

The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations (**Rule 64 (1)-(3) PCT**).
Therefore, in view of such prior art the subject-matter of the present application (**claims 1-26**) has to be regarded as being new (**Article 33(2) PCT**).

4. Inventive Step (**Article 33 (3) PCT**)

The **closest prior art** to evaluate the inventiveness of the present application is document **D1**. D1 discloses the immortalization of olfactory ensheathing glia (OEG) cells from rat, by transfecting OEG primary cultures from adult rats with a plasmid expressing a wild-type T antigen that is not oncogenic *in vivo*. The resulting immortalised cells show axonal regeneration properties.

The difference between the claimed subject-matter and the teachings of D1 is that the claimed method concerns the production of human OEG cells and it further comprises a last step wherein the oncogene responsible for the immortalisation is removed from the immortalised OEG cells.

Starting from **D1**, the underlying **technical problem** to be solved by the present application can be considered to lie in the provision of an alternative method to generate an unlimited source of OEG cells which are human and retain the axonal regeneration-promoting properties.

The **solution** provided by the Applicant to solve the above problem is a method of making OEG cells as defined in claim 1.

Regarding that **D1** is not concerned with tumorigenicity, because the immortalization system used therein is not oncogenic, and the fact that rodent cells are more easily manipulated and immortalized than human cells, D1 does not provide any indication that would teach the person skilled in the art to solve the above technical problem.

Documents **D2 and D3** both disclose a method to amplify scarce primary human cells *in vitro* to obtain them in a sufficient number for reimplantation in a patient, by reversible immortalisation by lentivector mediated transfer of specific genes.
However, there is no hint in these documents that the reversible cell immortalisation process described therein can be applied successfully to human OEG cells, and even less, that if applied to this cell type the cells obtained will show the desired axonal regeneration properties.

Moreover, regarding that **D3** clearly indicates that each human cell type presents difficulties in being reverse immortalised, this will be even more true for the special type of CNS cells such as the human OEG cells of the present application, quite different from those previously manipulated.
Therefore, the person skilled in the art would not have a reasonable expectation of success in applying the method of D3 to human OEG cells.

Document **D4** does not deal with human cells nor with reversible immortalisation.
Documents **D5 and D6** both deal with reversible immortalisation but are silent on the application of the process to human cells and do not mention human OEG cells.
None of the available prior art documents has shown the successful reverse immortalisation of a human CNS cell nor that the ability of the human OEG cells to promote axonal regeneration is conserved during the immortalisation and deimmortalisation process.
There is no indication in the prior art that points to the present invention.

The reverse-immortalisation of human OEG cells achieved in the present invention significantly reduces the risk of malignant transformation of transplanted OEG into patients.

Thus, the subject-matter of the present application (**claims 1-26**) is considered to involve an inventive step.

5. Industrial Applicability (**Article 33(4) PCT**)

5.1. The subject-matter of present **claims 1-7, 9-11, 13-19, and 21-26** is susceptible of industrial applicability as defined in **Article 33 (4) PCT.**

5.2. For the assessment of the present **claims 8, 12 and 20** on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

AMENDED CLAIMS

1. A method of making a population of human functional OEG cells for transplantation into a patient, which comprises:

a) providing a sample of primary human OEG cells;

b) immortalizing the OEG cells by transforming the OEG cells with a DNA construct comprising a removable DNA segment containing an oncogene or combination of oncogenes, thereby producing immortalised OEG cells;

c) growing the immortalised OEG cells;

d) selecting those immortalised OEG clonal cell lines which maintain their functional properties; and

e) removing the oncogene or combination of oncogenes from the immortalised OEG cells, the removal resulting in the production of the population of human functional OEG cells for transplantation into the patient.

2. The method of claim 1, wherein the oncogene or combination of oncogenes is made removable by flanking it with recombinase target sites, and the removing is accomplished by introducing into the immortalised cells a gene that is expressed to produce a recombinase that specifically recognizes the recombinase target sites.

3. The method of claim 2, wherein the recombinase is Cre recombinase and the recombinase target sites are loxP sites.

4. The method of claim 1, wherein the oncogene is the gene encoding SV40 large T antigen, the gene encoding telomerase catalytic subunit, the gene encoding Bmi-1 protein or any combination of said oncogenes.

5. The method of claim 1, wherein the removable DNA segment further contains a suicide gene, which encodes a gene product that enables destruction of the immortalised cells by an exogenous agent if the removable DNA segment is not removed from the cells.

6. The method of claim 5, wherein the suicide gene is a gene encoding herpes simplex virus thymidine kinase, and the cells are destroyed by exposure to gancyclovir if the removable DNA segment is not removed from the cells.

7. A population of human functional OEG cells produced by the method of claim 5.

8. A method of treating a patient for neural damage, comprising transplanting into the patient a sufficient quantity of the human OEG cells of claim 7 to provide promotion of neuronal regeneration in the neuronal injured region of the patient.

9. A method of making a population of human functional OEG cells for transplanting into a patient, which comprises:

a) providing a sample of primary human OEG cells;

b) immortalizing the OEG cells by transforming the OEG cells with a DNA construct comprising a removable DNA segment containing an oncogene or a combination of oncogenes, a selectable marker gene, and a gene encoding herpes simplex virus thymidine kinase, the genes together being flanked on either side by loxP sites;

c) growing the immortalised human OEG cells;

d) selecting those immortalised OEG clonal cell lines which maintain their functional properties; and

e) reversing the immortalization of the human OEG cells by removing the DNA segment from the immortalised OEG cells, the removing being accomplished by introducing into the immortalised OEG cells a gene encoding Cre recombinase to effect excision of the DNA segment at the loxP sites, the excision resulting in the production of the population of human functional OEG cells for transplanting into a patient.

10. The method of claim 9, wherein the oncogene is the gene encoding SV40 large T antigen, the gene encoding telomerase catalytic subunit, the gene encoding Bmi-1 protein or any combination of said oncogenes.

11. A population of functional OEG human cells produced by the method of claim 9.

12. A method of treating a patient for neural damage, comprising transplanting into the patient a sufficient quantity of the OEG cells of claim 11 to provide promotion of neuronal regeneration in the neuronal injured region of the patient.

13. An immortalised human OEG cell comprising a primary human OEG cell transformed with a DNA construct comprising two recombinase target sites that flank an oncogene or combination of oncogenes which confers immortalization to the OEG cell, wherein the immortalization is reversible by excision of the oncogene by cleavage at the recombinase target sites when the target sites are exposed to a recombinase that specifically recognizes the target sites.

14. The immortalised OEG cell of claim 13, wherein the recombinase target sites are loxP sites and the immortalization is reversible by Cre recombinase cleavage at the loxP sites.

15. The immortalised OEG cell of claim 13, wherein the DNA construct further comprises a selectable marker gene.

16. The immortalised OEG cell of claim 13, wherein the DNA construct further comprises a suicide gene, which encodes a gene product that enables destruction of the immortalised OEG cell by an exogenous agent if the oncogene is not removed from the cells.

17. The immortalised OEG cell of claim 16, wherein the suicide gene is a gene encoding herpes simplex virus thymidine kinase, and the exogenous agent is gancyclovir.

18. A cell line comprising a population of the immortalised human OEG cell of claim 13.

19. A reverse-immortalised OEG human cell that is functional upon transplantation into a patient, produced by exposing the DNA construct within the immortalised human OEG cell of claim 13 to a recombinase that excises the oncogene or combination of oncogenes by cleavage at the recombinase target sites.

20. A method of treating a patient for neural damage, comprising transplanting into the patient a sufficient quantity of the reverse-immortalised OEG human cells of claim 19 to provide promotion of neuronal regeneration in the neuronal injured region of the patient.

21. A cell library comprising a collection of reverse-immortalised OEG human cells prepared according to the methods of any one of claims 1 to 6 or 9 to 10.

22. A reverse immortalised functional human olfactory ensheathing glia (OEG) cell line.

23. A cell line according to claim 22, for use in a method of therapy.

24. A cell line according to claim 22, for use in promoting neuronal regeneration.

25. A pharmaceutical composition comprising a human cell line as defined in claims 22-24, and a pharmaceutically acceptable carrier.

26. The use of reverse-immortalised human olfactory ensheathing glia cells as defined in claims 7, 11, 19 or 22 in the preparation of a medicament for treating neuronal damage.